BioHydrogen (BioH2) Consortium to Advance Fermentative H_2 Production

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National Renewable Energy Laboratory
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DOE Hydrogen Program
2024 Annual Merit Review and Peer Evaluation Meeting

Project ID: P179

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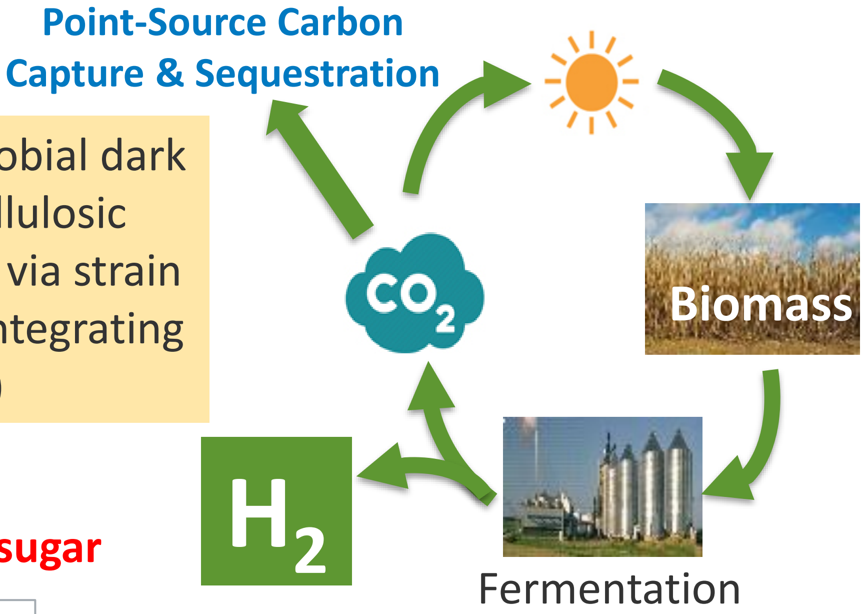
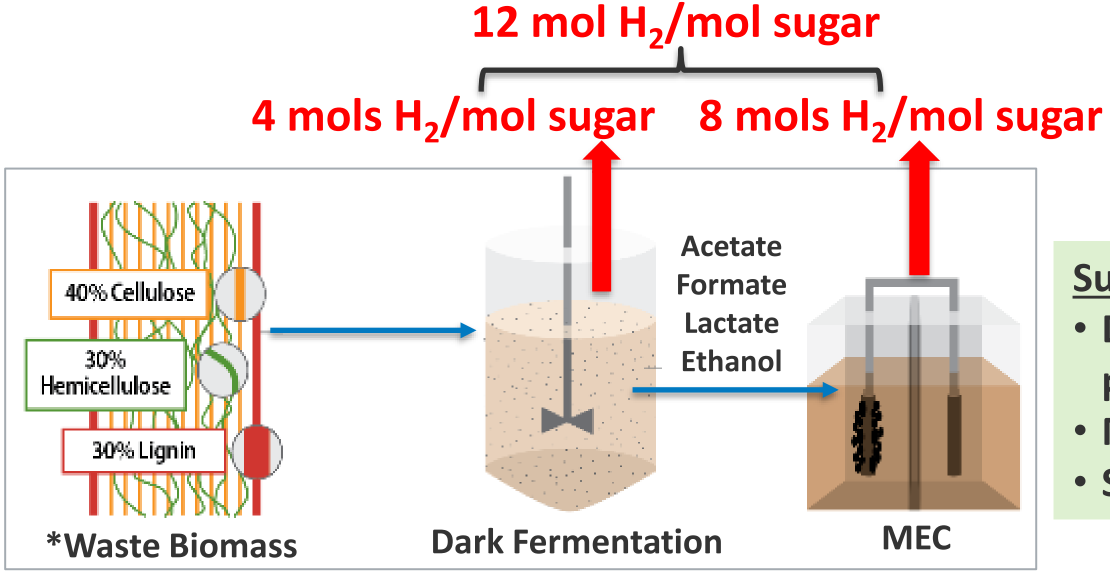
Project Goal

Overall Objective: Develop a carbon-neutral, microbial dark fermentation technology to convert waste lignocellulosic biomass into H_2 with a production cost < \$2/kg-H_2 via strain engineering, bioprocess design for scale-up, and integrating fermentation with microbial electrolysis cell (MEC)





**Solid, size reduction, not chemically pretreated*

Successful Outcomes:

- Decentralized, economic, and green H_2 production with decarbonization potential
- Monetize organic wastes for H_2 production
- Support rural & developing economies

Overview

Timeline and Budget

- Project start date: 10/1/2018
- FY23 DOE funding: $1.3M
- FY24 planned DOE funding: $1.2M
- Total DOE funds received to-date *$6.5M

  *Dollars received by the consortium since project start

	FY19	FY20	FY21	FY22	FY23	FY24
NREL	$485K	$600K	$600K	$300K	$780K	$700K
LBNL	$200K	$200K	$150K	$150K	$180K	$180K
PNNL	$200K	$200K	$200K	$150K	$180K	$180K
ANL	$200K	$125K	$125K	$ 75K	$180K	$150K
Total	$1.1M	$1.1M	$1.1M	$675K	$1.3M	$1.2M

Barriers

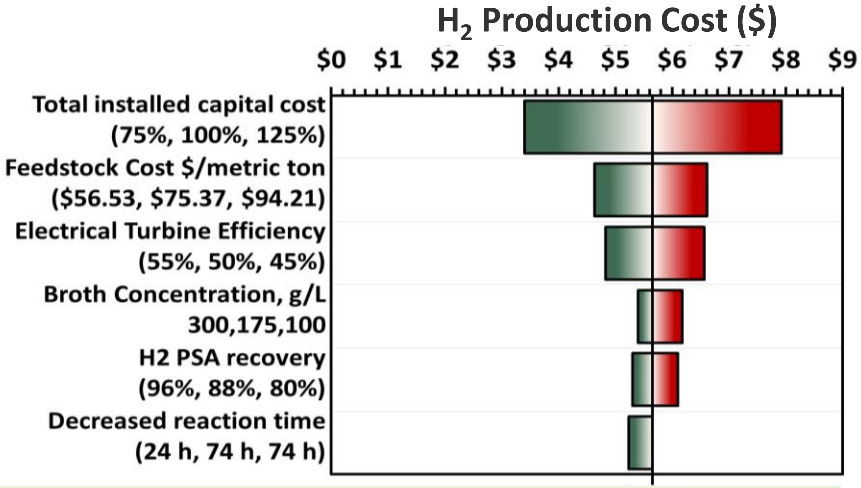
- Capital cost
- Feedstock cost (AY)
- H_2 molar yield (AX)

Partners

- Project lead:
  Katherine Chou, Ph.D. (**PI, NREL**)
- Co-PIs: Eric Sundstrom, Ph.D. (LBNL)
  Alex Beliaev, Ph.D. (PNNL)
  Amgad Elgowainy, Ph.D. (ANL)
- Lawrence Berkeley National Lab (LBNL)
  Pacific Northwest National Lab (PNNL)
  Argonne National Lab (ANL)

Relevance & Impact

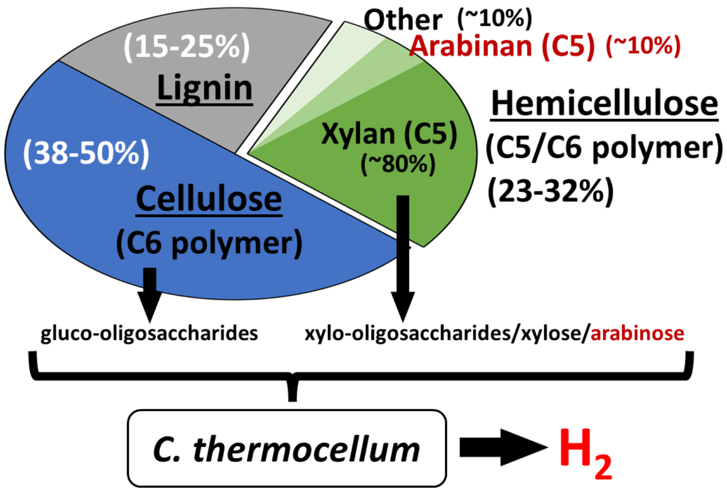
A collaborative team of scientists from 4 national labs whose experts builds a strong foundation in addressing knowledge gaps and technical barriers for long-term success toward meeting the H_2 production cost goal (< $2/kg H_2).



- Achieved bio-H_2 production cost reduction **from > $58/kg-$H_2$ to ~$12.4/kg H_2 (TRL 2-4)**
  - R&D priorities and cost reduction strategies:
    - lower bioreactor (CAPEX) & feedstock costs via reduced bioreactor footprint, increased H_2 yield, high-loadings of biomass fermentation, efficient biomass deconstruction, utilization, conversion; tax credits; cost-advantaged feedstocks
- Use **solid** waste biomass directly
- Reduced electricity use (**by more than half**) for bio H_2 relative to PEM water electrolyzer
- Remarkable decarbonizing potential unique to biological H_2
  - Excellent niche for hard-to-decarbonize sectors
- Basic & applied R&D remains key enablers for bio-H_2

Approach: Task 1. Improve biomass utilization and conversion (i.e., H_2 Yield) via *Clostridium thermocellum* strain development (NREL)

Engineer the cellulose-degrading microbe to co-metabolize hemicellulose, pentose sugars: xylose & arabinose (FY 23/24)



Ferment all the sugars to H_2 in one bioreactor: lowering both feedstock and reactor costs.

Improve sugar utilization & H_2 yield (e.g., for a given amount of feedstock)

- **1926** — *C. thermocellum* discovered
- **1926-2015** — Wild *C. thermocellum* (***Δhpt***) utilizes cellulose (C6) but not hemicellulose (C5)
- **2016-2018** — NREL genetically modified strain (***xylAB***) to co-utilize xylose (C5)
- **2018-2019** — NREL evolved the bacteria for improved growth and H_2 production rate on xylose (a hemicellulose sugar) (strain ***19-9***)
- **2020-2022** — NREL enabled the co-utilization of hemi-/cellulose (created strain ***BXint***) to break down xylose-oligomers
- **2023-2024** — NREL expressed foreign genes to utilize **arabinose** (a hemicellulose sugar), created ***BXintARAint*** strain

Approach: Safety Planning and Culture

Required to submit a safety plan to the Hydrogen Safety Panel (HSP)?

No, a safety plan is not required for this project.

Prioritizing Safety & Analyzing Hazards

- Hazard Analysis Reviews (HAR) were recently performed and updated to assess, identify, and control for risks involved in all research activities during labs relocation
- All researchers are compliant with a Required Training Plan (RTP) tailored for all the planned lab and bench scale experimentations
- All research activities are conducted in compliance with ESH&Q and Biosafety guidance

Incidents and near-misses

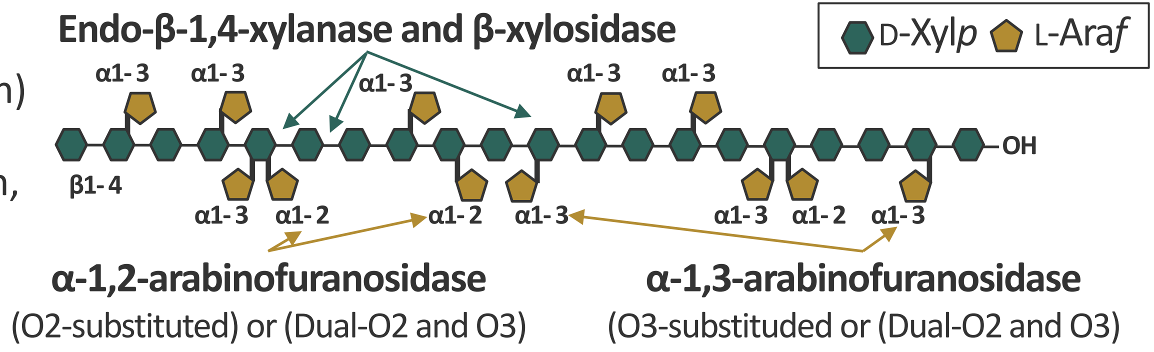
- Compressed gas cylinder safety is regularly discussed
- Potential needlesticks during anaerobic bacterial cultivation are discussed regularly for prevention

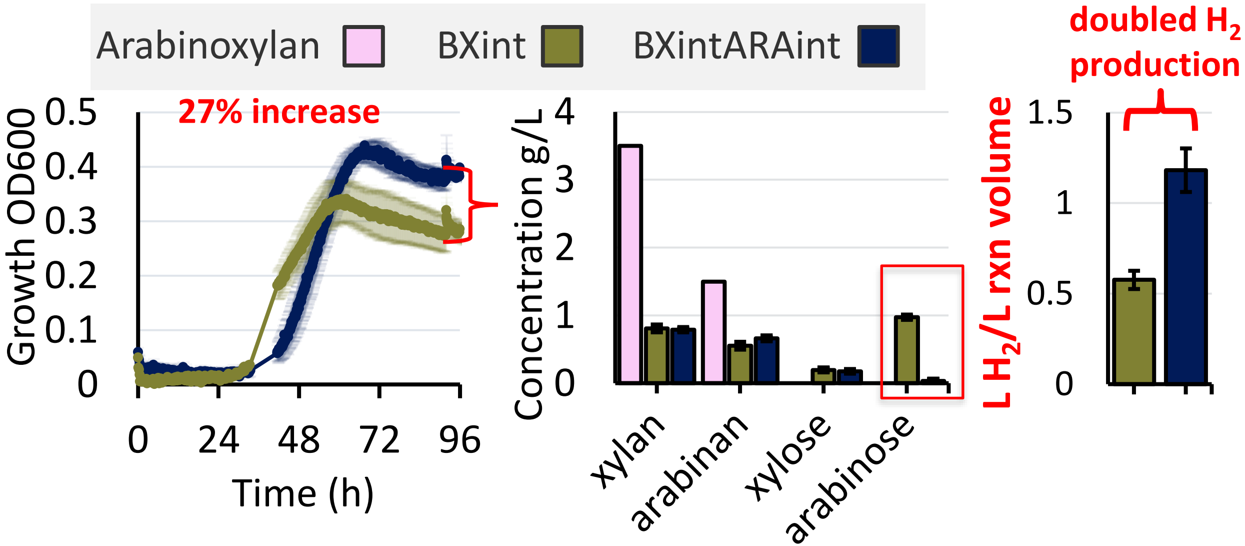
Best Safety Practice /Lessons Learned

- 5% H_2 forming gas (5% H_2, balance N_2) is used instead of 10% H_2 forming gas
- Research SOPs are reviewed regularly with close ESH&Q oversight
- Proper microbial decontamination procedures are practiced
- Close mentoring of new research staff and interns are practiced

Accomplishments & Progress Task 1: Doubled H_2 production from arabinoxylan biomass via an engineered strain (NREL, FY23 Q3)

- Arabinose genes integrated into *C. thermocellum* genome (BXintARAint strain)
- Test if engineered strain has the enzymes to deconstruct and consume arabinoxylan, the main hemicellulose of herbaceous plants.





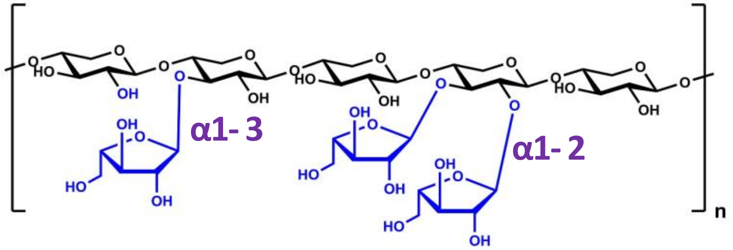
BXintARAint strain

- Grew to higher density, 27% increase.
- Deconstructed similar amounts of arabinoxylan as BXint but consumed the arabinose.
- **Produced 105% more H_2.**

All strains left 20% xylan and 40% arabinan intact.

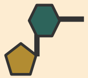
Accomplishment: Task 1. Identified recalcitrant bonds in biomass toward full utilization (NREL, FY24 Q1) using Nuclear Magnetic Resonance (NMR)

NMR data is used to guide future strain engineering toward complete biomass utilization



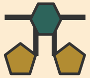
Arabinoxylan is xylan decorated with α1,2- and α1,3-linked arabinose

- An unbroken bond physically blocks the enzymes to access other bonds and sugars
- The impact of each unbroken bond is amplified at higher loadings and scale-up

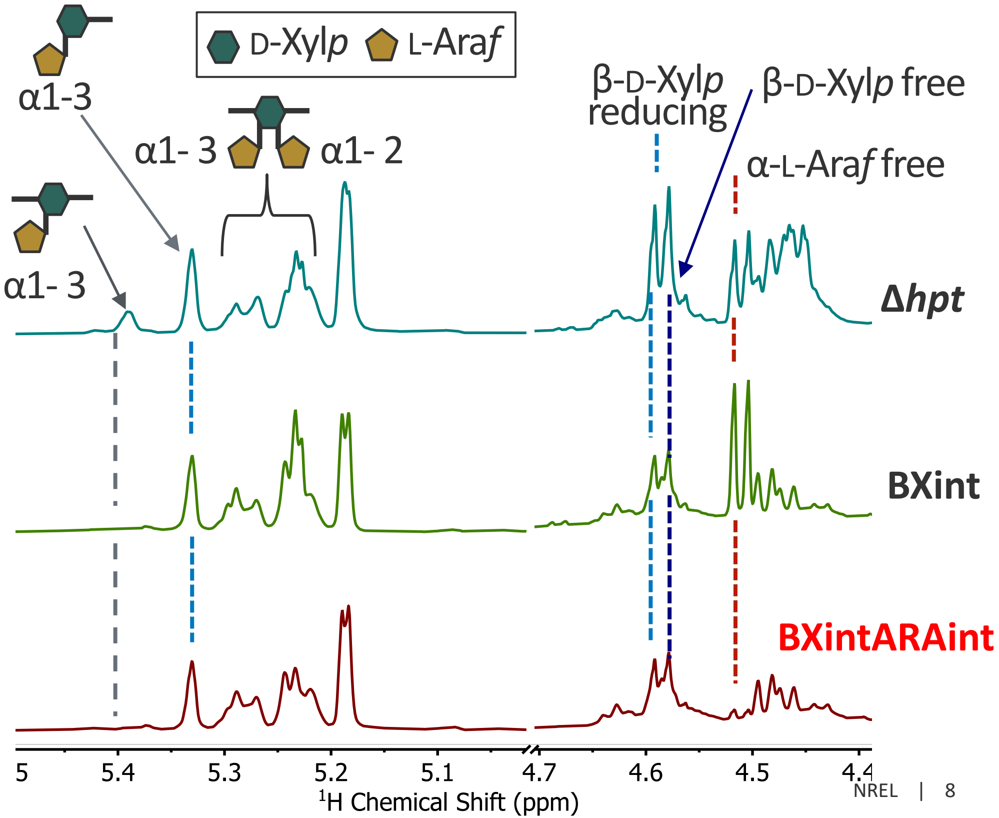


α1-3

α1,3-linked arabinose at the **non-reducing end** prevents deconstruction.

α1- 3  α1- 2

Dual-linked arabinose is blocked from deconstruction.

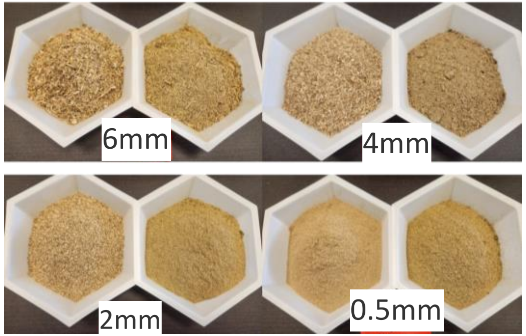


Approach: Task 2. High-Solids Bioreactor Development (LBNL)

Approach: Optimize H_2 production from milled corn stover (MCS) under high solids loading conditions, and demonstrate feasibility of scale-up to >15 L production volumes (Task 2)

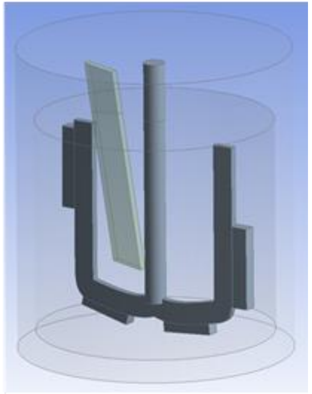
- Compare H_2 production and solubilization of biomass (glucose, xylose) for *C. thermocellum 19-9* across a range of milled corn stover particle sizes (0.5, 2, 4, 6 mm)
- Complete commissioning of a 50 L bioreactor system featuring anchor impeller, flow breaker, and vacuum gas removal

Milled Corn Stover



left: before fermentation
right: post fermentation (finer particles)

Customized bioreactor for scale-up



ABPDU fermentation suite is equipped with Rushton and anchor impeller bioreactors, process mass spectrometer, and a 50 L scale-up reactor with customized, high-solids mixing geometry

Accomplishments/Progress: Task 2. Achieved 69% solubilization of total biomass carbohydrates from >45 g/L milled corns stover biomass (LBNL)

Batch fermentation (1.5L) of milled corn stover (MCS)

(45-75 g/L)

Solubilization	FY22	FY23
Glucan	52%	**66%**
Xylan	61%	**73%**
Total Carb.	55%	**69%**

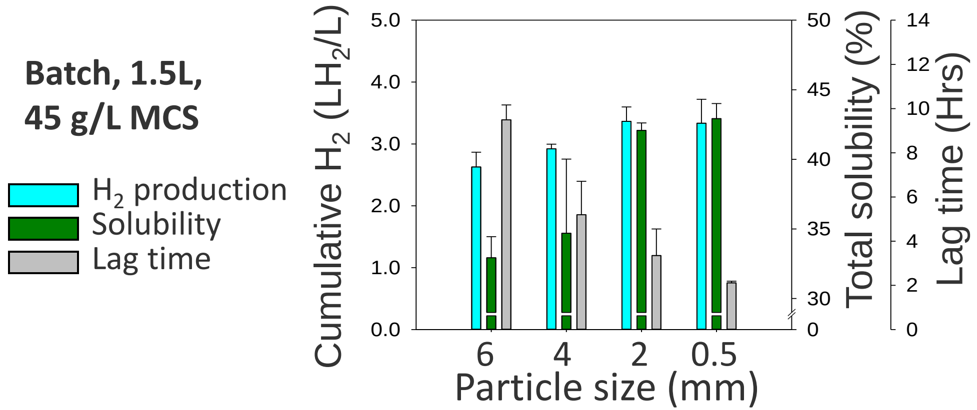
Parameters explored:
- ○ Particle sizes
  - FY22: 2-6mm
  - FY23: **0.5-2 mm**
- ○ Mixing (45-**100** rpm)
  Faster mixing is beneficial

Scale-Up (50L) of MCS Batch Fermentation

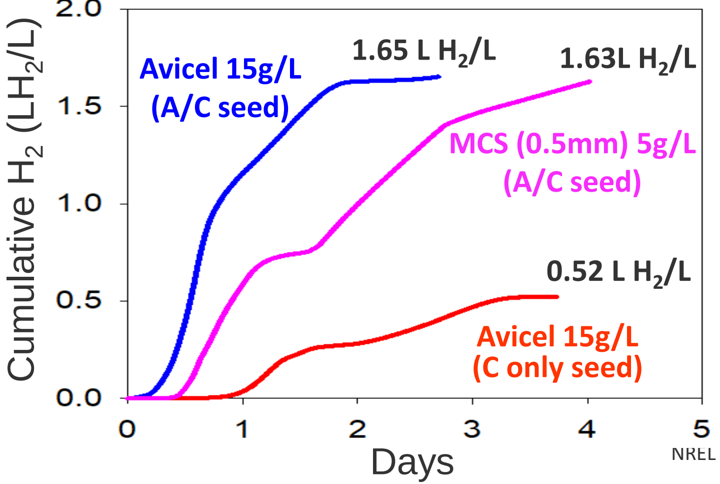
- ➢ **Commissioning of a customized 50 L bioreactor for high solids loading**
  - ○ **anchor style impellers & flow breakers**
- ➢ **Tested and improved seed culture acclimation strategies for scale-up**
  - ○ **achieved 15% more H_2 yield than a previous MCS fermentation (0.326 vs 0.285 L H_2/g biomass)**

H_2 yield vs. particle size (milling cost)

Batch, 1.5L, 45 g/L MCS



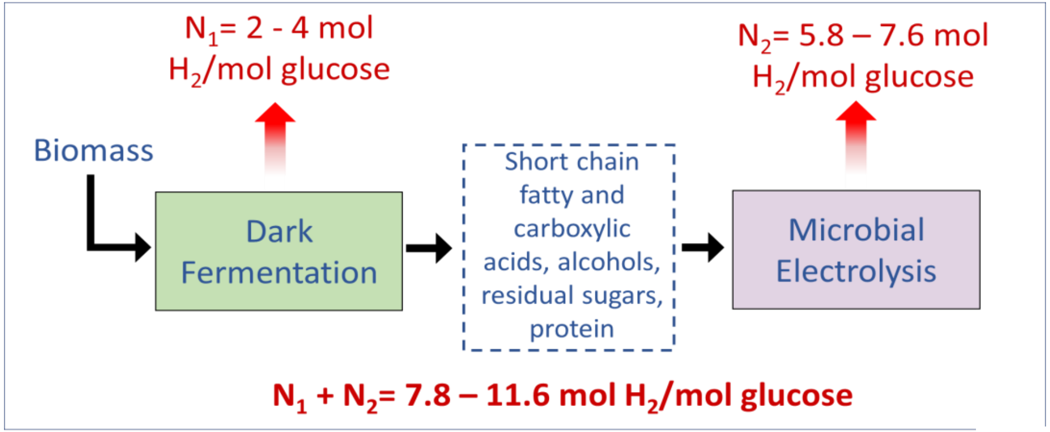
A: Avicel, C: Cellobiose



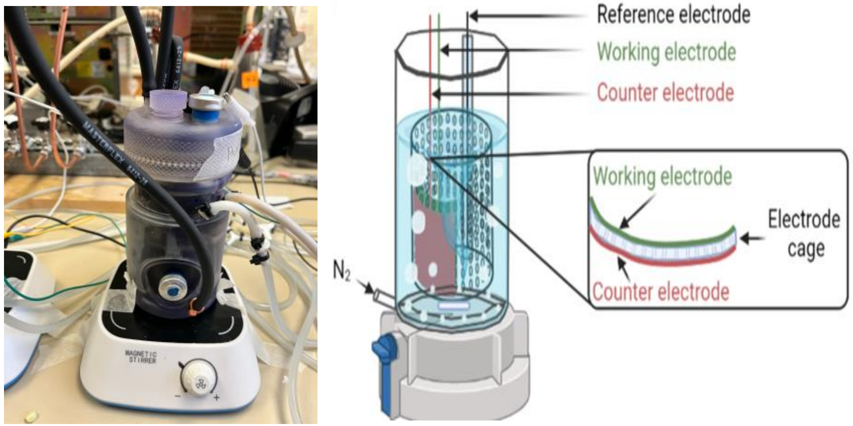
Approach: Task 3. Microbial Electrolysis Cell (PNNL)

Approach: Design MEC process integrated with dark fermentation (Tasks 1 & 2) for conversion of the fermentation effluent to H_2 using robust exo-electrogenic microbes & consortia

- Deploy robust and controllable exo-electrogenic consortia with broad metabolic capacity to increase H_2 production from fermentation effluent
- Rationally design continuous MEC process for conversion of lignocellulosic fermentation effluent (e.g., organic acids, alcohols, proteins, sugars) to H_2 with increased efficiencies and productivities.



Process flow diagram of the integrated fermentation-MEC process for H_2 production from waste biomass

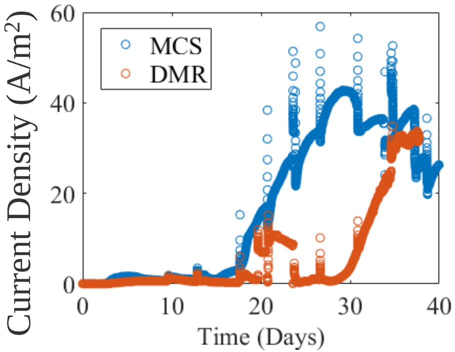


H_2 production in single-chamber MEC's using effluent from high-solid loading DMR fermentation

Accomplishments and Progress: Task 3. Achieved sustainable MEC operation at 30 A/m^2 on both DMR and MCS effluent (PNNL) & analysis of microbial community of cathode and anode of MEC

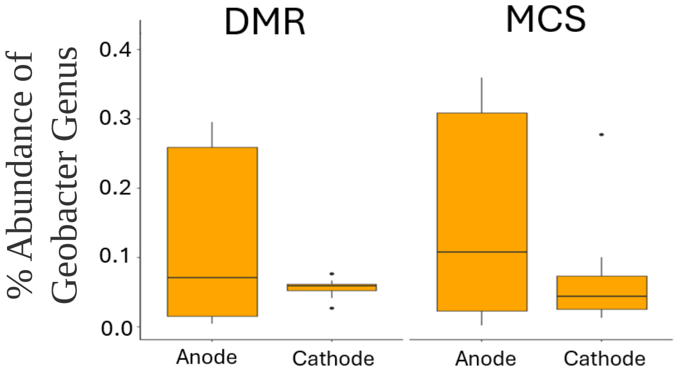
FY23 Q4 Milestone: Optimize the performance of single-chamber MEC using MCS effluent from high- solid load fermentation to achieve ≥ 30 A/m^2 and ~1 L H_2 / L reactor volume/day	Complete July 2023

MEC Performance



- New 3D-printed MECs were designed for: Wall-to-wall bracing to eliminate flexing, rounded plane intersections to prevent cracking, & withstand 6 months of stress testing
- MECs were inoculated with anaerobic granules from WWTP and fed with milled corn stover (MCS, 15 g/L) and DMR (30 g/L, chemically pretreated) biomass
- Sustained current densities > 30 A/m^2 were obtained on effluent from high-solid loading MCS fermentation process

Microbial Community Analysis

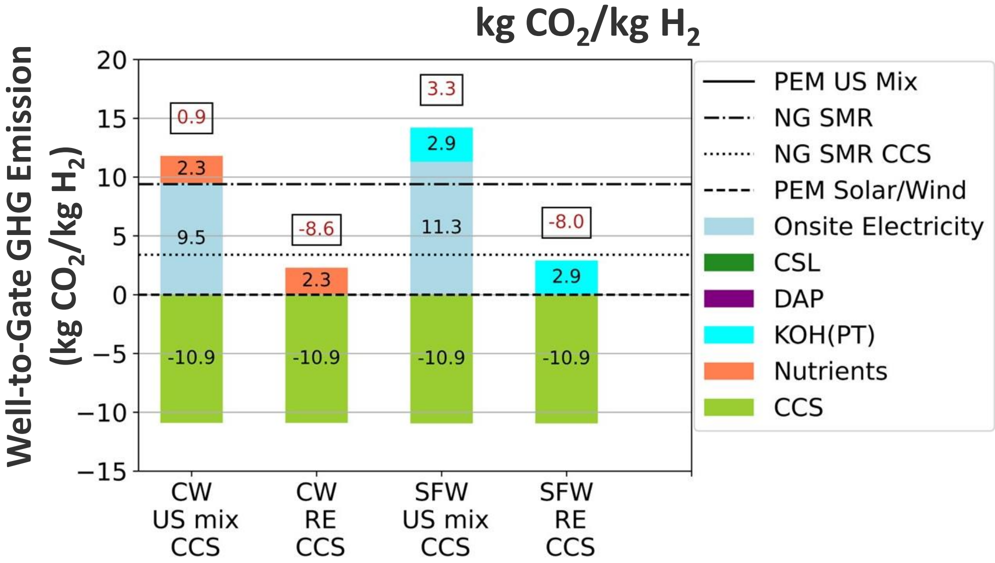


- Significant enrichment in exoelectrogenic Geobacter spp. was observed in MECs that operated at high current densities (>30 A/m2) over extended periods (>30 days).
- Detected a concurrent increase in H_2 scavenging species abundance (methanogens, acetogens, sulfate-reducers) on both cathode and anode

Approach: Task 4. Conduct TEA and LCA for the modeled process featuring cost-advantaged feedstocks for bioH_2 production (ANL)

Use TEA (Aspen Plus) and LCA (GREET) to set research targets, guide research directions and suggest system design to achieve cost targets and reduce life cycle greenhouse gas (GHG) emission

- **Cost Advantaged Feedstocks –** waste streams providing a revenue incurred from its disposal (e.g., tipping fee, wastewater discharge fee).
- Proof-of-Concept: Wastewater from cheese whey (CW) production and solid food waste (SFW) are used to assess the potential reduction in feedstock and overall bio-H_2 production costs.
- GHG emission primarily comes from grid electricity. Electricity usages are 20.4 kWh/kg for CW wastewater and 24.2 kWh/kg for SFW, less than PEM (55.5 kwh/kg).
- With wind/solar electricity and CCS, the net GHG emissions are negative, potentially qualifying for IRA 45V tax credit of \$3.0/kg H_2.
- Bio-H_2 can potentially qualify for 45Q credit, which is less beneficial than 45V.



CCS: Carbon Capture & Sequestration; RE: Renewable energy (solar/wind); CW: Cheese whey; SFW: Solid food waste

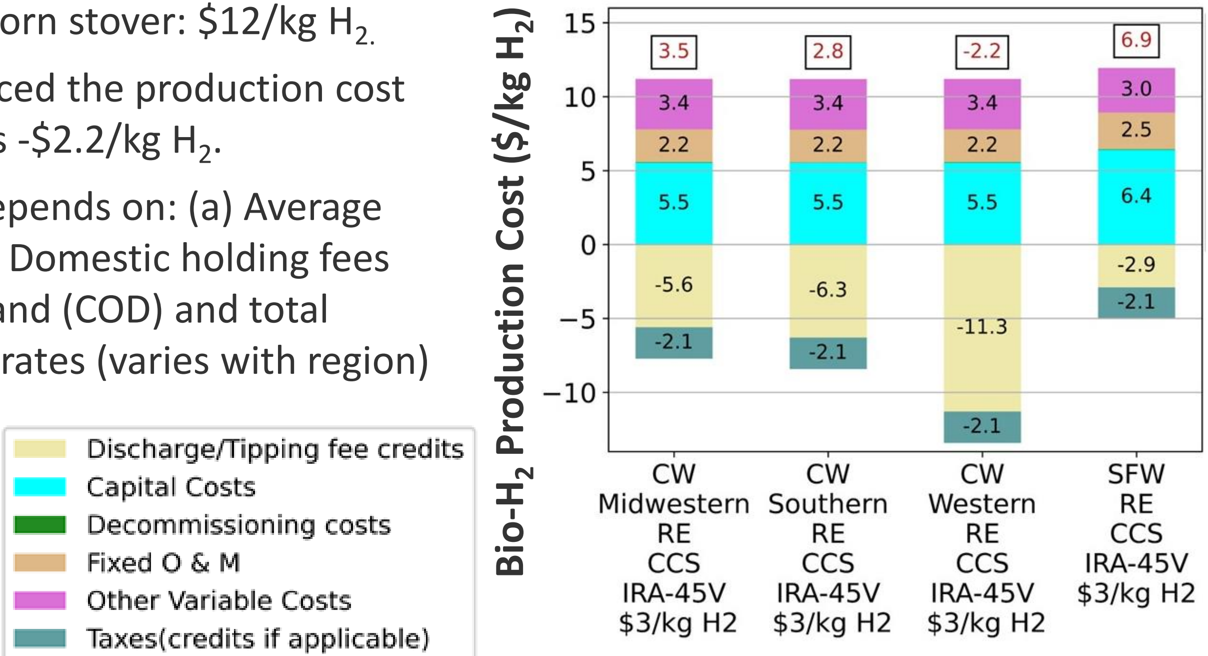
Based on a 50 MT/day bio-H_2 production plant

Accomplishments & Progress: Task 4. Identified bio-H_2 cost reduction opportunities using cost-advantaged feedstocks and tax credit (ANL)

FY23 Q4 Milestone: Conduct TEA and LCA for the modeled process featuring cost-advantaged feedstocks (CW and SFW) identified in previous quarters for bio-H_2 production.	**Sept 2023, Complete**

TEA of Cheese Whey (CW) wastewater or Solid Food Waste (SFW) to 50 MT/day bio-H_2

- Current Bio-H_2 production from corn stover: \$12/kg H_2.
- Cost advantaged feedstocks reduced the production cost to below \$6.9/kg H_2 and as low as -\$2.2/kg H_2.
- CW wastewater discharge fees depends on: (a) Average volume discharge fees (fixed); (b) Domestic holding fees (fixed); (c) Chemical oxygen demand (COD) and total suspended solids (TSS) discharge rates (varies with region)
- SFW: the municipal solid waste tipping fee is \$53/MT. Assume 60% of biomass utilization leads to a resultant tipping fee of \$32/MT SFW = -\$2.9/kg H_2.



RE=Renewable Energy (solar/wind)

Responses to Reviewers' Comments

Progress toward lower overall H_2 production cost: On-going fermentation R&D to completely utilize biomass will allow greater biomass deconstruction/solubilization, therefore better biomass utilization, which leads to higher loading (>50-100 g/L) and further reduce the cost below \$12.4/kg H_2. TEA in FY23 also provided the projection that using alternative, cost-advantaged feedstock will provide revenues to substantially lower the production cost to < \$0/kg H_2 depending on the feedstock types and region. (Note: This project leverages industrial support from Southern California Gas Company to have demonstrated bio-H_2 production from a range of cost-advantaged feedstocks to augment the scope of DOE funding). FY24 is also set to explore potential revenue from upgrading residual lignin to higher value products rather than burning (current baseline).

*A summary of cost reduction opportunity from \$12.4/kg H_2 to \$3.3/Kg H_2 before the use of cost-advantaged feedstocks and lignin upgrading is provided as an additional slide.

Team Integration: NREL strains developed are tested for higher loadings and larger scale (50L) at LBNL, and such results from the strains performance at scale informs further strain development. This process is iterated to cross-inform bioreactor and strain development. The wastewater at the end of fermentation from LBNL/NREL is saved and shipped to PNNL for MEC development to inform optimal fermentation conditions that leads to high H_2 yield by MEC, as well as identifying potential inhibitors to MEC operation. While we are keen to setup tests for a truly integrated fermentation-MEC process in the future, strains developed in real time is used in scale-up, and real wastewater from scaled up is used for MEC.

Avoiding N_2-gas in dark fermentation: Fermentation at scale is setup to test a slightly negative pressure (mild vacuum) rather than nitrogen gas sparging to draw the H_2 gas out.

TEA model: The model is based on real experimental data of percentage of biomass solubilization/utilization, biomass loading (50-100 g/L), and the best current density achieved by MEC (66 A/m^2). Higher biomass loading is projected to reduce H_2 production cost further. Note that the best MEC current density was not achieved using the milled biomass fermentation wastewater and much of the R&D is underway to achieve high current density with this complex feedstock without chemical pretreatment. In addition, TEA model is setup to compare the trade-offs between the cost of milling versus H_2 yield.

DEIA/Community Benefits Plans and Activities

This projects does not have a Diversity, Equity, Inclusion, and Accessibility (DEIA) plan or Community Benefits Plan (CBP), so this slide is optional.

Energy & Environmental Justice

Waste streams are often disproportionally channeled into more disadvantaged communities. This project addresses issues surrounding organic wastes and diverts them for bio-H_2/energy production, which can empower local, farming, and developing communities.

Collaborations with MSIs

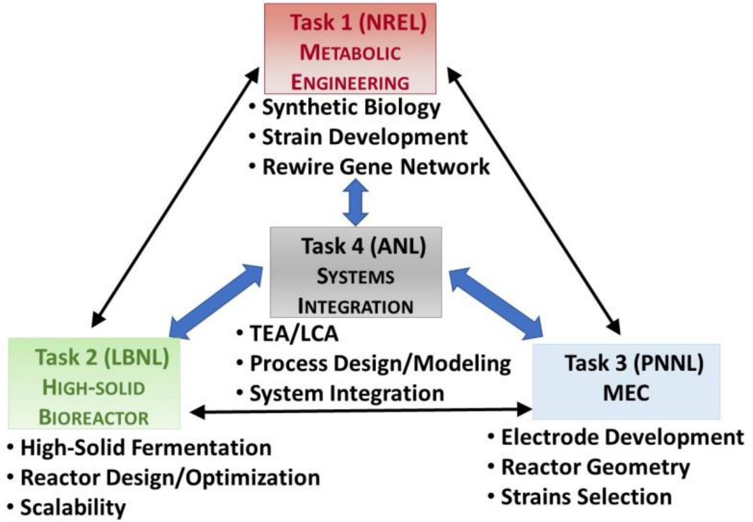
NREL is collaborating with Dr. Harvey Hou, a Professor in forensic science program at Alabama State University (a HBCU) to identify unique fingerprints of *Clostridium thermocellum*.

Community Engagement

NREL PI and a staff researcher conducted a STEM education outreach event at Trailside, a metro Denver underserved elementary school

NREL researcher Eric Schaedig conducting life microscopy session to show microbes living in a drop of pond water

Collaboration & Coordination



- **Task 1. Strain Development and Improvement (NREL)**
  - NREL sets direction and coordinates efforts between labs
  - Develop and test strains to improve H_2 production
  - Send strains to LBNL for testing in high solids fermentation
  - Leverage BETO investment in biomass and Office of Science BER investments (UCLA, Oak Ridge National lab) in *C. thermocellum* physiology and gene regulation.
- **Task 2. High-solids Bioreactor Development (LBNL/NREL)**
  - Develop and co-optimize bioreactors for high solid loadings and supply fermentation effluent to PNNL.
  - Received modified strains from NREL for testing.
- **Task 3. Microbial Electrolysis Cell (PNNL)**
  - Collaborate with Washington State University – bioelectrical system design
  - Optimizing fermentation-MEC integration with NREL/LBNL and improve the H_2 molar yield
- **Task 4. System Integration, TEA and LCA (ANL)**
  - Develop and use TEA/LCA to set research targets and guide research directions
  - Work closely with all other tasks to explore production cost reduction opportunities

Remaining Challenges and Barriers

Tasks 1. Strain Development and Improvement (NREL)

- H_2 yield is compromised due to incomplete utilization of the biomass
- Physical bonds linking the sugars block enzyme accessibility for hydrolysis
- The impact of each unbroken bond is amplified at higher loadings and scale-up

Task 2. High-solid Bioreactor Development (LBNL)

- Overall conversion efficiency declines at high solids loading (bulk viscosity) and larger particle sizes (likely lower accessibility to biomass sugars)
- Nitrogen gas is currently used for H_2 removal and ensure anaerobic conditions. Full deployment will require an alternative (e.g., vacuum) to avoid costly gas separations.

Task 3. Microbial Electrolysis Cell (PNNL)

- Improve conversion efficiencies and H_2 molar yield on milled biomass effluent
- Improve electron transfer in electrogenic biofilms and at microbe-electrode interface

Task 4. System Integration, TEA and LCA (ANL)

- TEA results identify MEC current density drives the capital costs.

Proposed Future Work

Note: Any proposed future work is subject to change based on funding levels.

Task 1. Strain Development and Improvement (NREL)

- Recombinantly express additional enzymes to break chemical bonds in biomass to unlock more sugars (arabinose, xylose, glucose) for utilization and increased H_2 yield
- Improve strains for better biomass deconstruction, utilization, and H_2 yield at higher loadings

Task 2. High-solid Bioreactor Development (LBNL)

- Eliminate separation costs associated with nitrogen sparging via implementation of a vacuum-based gas removal system
- Demonstrate process robustness via long-term continuous operation with milled corn stover biomass

Task 3. Microbial Electrolysis Cell (PNNL)

- Optimization of milled biomass wastewater conversion to achieve higher H_2 production rates
- Characterization of anodic biofilm enriched consortium to enable rational design and control

Task 4. System Integration, TEA and LCA (ANL)

- Identify/explore additional cost reduction pathways, e.g., lignin upgrading, MEC design, low-cost feedstock
- Deep dive into understanding the trade-offs between energy costs associated with biomass size-reduction strategies (e.g., milling) vs. H_2 yield

Summary

Task 1. Strain Development and Improvement (NREL)

- **Successfully engineered a strain to utilize arabinose, a hemicellulose sugar, toward complete biomass utilization**
- **Doubled H_2 yield from arabinoxylan, a model hemicellulose using an engineered (BXintARAint) strain**
- **Identified remaining and recalcitrant bonds in biomass sugars to guide future strain engineering efforts**

Task 2. High-solid Bioreactor Development (LBNL)

- Achieved **66.4% glucan solubilization and 73.3% xylan solubilization** at 75 g/L solids loading with milled corn stover via optimization of particle size, culture acclimation, and bioreactor mixing conditions
- Successfully **transitioned from 1.5 L high solids bioreactors to a newly commissioned 50 L bioreactor** system, achieving 1.63 L/L H_2 production from 5 g/L milled corn stover, at a yield of 0.326 L H_2 / g biomass.

Task 3. Microbial Electrolysis Cell (PNNL)

- New single-chamber design significantly improves MEC performance (improved robustness, reduced resistance)
- Achieved **$\geq$ 30 A/m^2** using fermentation wastewater generated **with complex, real biomass without chemical pretreatment (milled corn stover).**

Task 4. System Integration, TEA and LCA (ANL)

- **Evaluated the potential and provided a proof-of-concept of using cost-advantaged feedstocks to reduce cost.**

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Steve Singer, Ph.D.

PNNL
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Thank You

www.nrel.gov

Publication Number

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